

HMG-CoA reductase, cholesterol 7 α -hydroxylase, LDL receptor, SR-B1, and ACAT in diet-induced syndrome X

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Background. Long-term consumption of Western diets can lead to acquired syndrome X, which presents with obesity, insulin resistance, hypertension, hyperlipidemia, and risk of atherosclerotic cardiovascular disease. While plasma lipid abnormalities in syndrome X have been well characterized, their molecular basis remains unclear. This study explored potential mechanisms of hypercholesterolemia in diet-induced syndrome X.

Methods. Female Fischer rats were fed a high-fat, refined-carbohydrate (sucrose) diet (HFS) or standard rat chow (low-fat, complex carbohydrate, LFCC) for 20 months. Plasma lipids and hepatic tissue mRNA, protein, and/or activities of the key enzymes and receptors involved in cholesterol metabolism were determined.

Results. The HFS group exhibited hypertension, hyperlipidemia, insulin resistance, obesity, significant down-regulation of hepatic cholesterol 7 α -hydroxylase (the rate-limiting step in cholesterol catabolism) and low-density lipoprotein (LDL) receptor (LDL-R, the primary pathway of LDL clearance). In contrast, hepatic tissue acyl-coenzyme A:cholesterol acyl-transferase (ACAT-2, the primary enzyme involved in intracellular esterification of cholesterol) and scavenger-receptor class B, type 1 (SR-B1 or HDL receptor) were up-regulated. While 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA expression was increased, its protein abundance and activity were unchanged, and HMG-CoA reductase-to-cholesterol 7 α -hydroxylase ratio was increased in HFS-fed animals.

Conclusion. Hypercholesterolemia in diet-induced syndrome X is associated with depressed cholesterol 7 α -hydroxylase, diminished LDL-R, elevated ACAT, and increased HMG-CoA reductase-to-cholesterol 7 α -hydroxylase ratio. These findings point to impaired hepatic catabolism and uptake of cholesterol and inappropriate cholesterol production capacity as the underlying causes of hypercholesterolemia in rats with diet-induced syndrome X.

Key words: syndrome X, obesity, lipid disorders, insulin resistance, diabetes, cholesterol.

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The metabolic syndrome, also known as syndrome X, refers to a constellation of abnormalities which include insulin resistance, hypertension, endothelial dysfunction, obesity, dyslipidemia, oxidative stress, inflammation, propensity for thromboembolism, and arteriosclerotic cardiovascular disease [1–3]. The prevalence of the metabolic syndrome in westernized societies has risen at an alarming rate, affecting ~23% of the general population and ~43% of those over 60 years of age in the USA [4]. A sedentary lifestyle and unhealthy diet (high saturated fat and refined carbohydrates) are largely responsible for the ever-increasing occurrence of the metabolic syndrome and its complications, namely type II diabetes and cardiovascular disease. In fact, genetically normal rats fed a high-fat, high-sucrose diet (HFS) for extended periods exhibit nearly all features of the metabolic syndrome, pointing to the central role of diet in the pathogenesis of this syndrome [5–9].

Dyslipidemia in syndrome X is marked by elevation of plasma triglycerides, small-dense LDL, as well as a high total cholesterol to high-density lipoprotein (HDL) ratio, denoting an atherogenic profile. In an earlier study, we showed marked down-regulation of skeletal muscle lipoprotein lipase and very-low-density lipoprotein (VLDL) receptor in rats with diet-induced syndrome X [10]. Acquired deficiencies of lipoprotein lipase and VLDL receptor, which are the principal pathways for clearance of triglyceride-rich lipoproteins from the circulation, can, in part, account for the elevation of plasma triglyceride and VLDL in rats with diet-induced metabolic syndrome. Moreover, the marked down-regulation of lipoprotein lipase (LPL) and VLDL receptor in the skeletal muscle, coupled with an elevation of adipose tissue LPL activity, points to a shift in fatty acid metabolism from energy consumption in muscle to energy storage in adipocytes. This phenomenon can contribute to the development of obesity in rats with diet-induced syndrome X despite the absence of hyperphagia [10].

We hypothesized that hypercholesterolemia associated with long-term consumption of an HFS diet may reflect altered hepatic expression of the key enzymes and receptors involved in cholesterol metabolism in rats with diet-induced syndrome X. Accordingly, mRNA, protein abundance, and/or enzymatic activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, cholesterol 7 α -hydroxylase, the rate-controlling enzyme in cholesterol catabolism to bile acids, low-density lipoprotein (LDL) receptor (LDL-R), the primary pathway of LDL clearance from the plasma, scavenger-receptor class B, type 1 (SR-B1), the main pathway of hepatic HDL delipidation, and acyl-coenzyme A:cholesterol acyltransferase (ACAT-2), the primary enzyme responsible for intracellular esterification of cholesterol, were determined in the liver of rats after long-term HFS diet consumption. The results were compared with those obtained in the control rats fed standard rat chow (LFCC) diet.

METHODS

Animals and diets

All protocols were conducted in accordance with the University of California, Los Angeles, Animal Research Committee. Inbred female Fischer rats were obtained from Harlan Sprague-Dawley, Inc. (San Diego, CA, USA), at 2 months of age. We have used this rat model in our previous studies, as the female Fischer rat normally shows little weight gain beyond the maturation phase [9, 10]. The rats were randomly assigned to either the LFCC or HFS diet. They were housed 4 per cage with a 12-hour light cycle starting at 0700 hours at 75 to 76°F, and were fed ad libitum with bowls placed in the cages to assure that all animals had access to the food. The diets were prepared in powder form by Purina Test Diets, Inc. (Purina, Richmond, IN, USA) and contained a standard vitamin and mineral mix and all essential nutrients. The LFCC diet (Purina 5001) is low in saturated fat and contains mostly complex carbohydrate (starch), while the HFS diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of corn oil) and high in refined-sugar (sucrose) as previously described [7]. After 20 months, and an overnight fast to eliminate any confounding effects of the last meal, the animals were anesthetized with chloral hydrate (250 mg/kg ip), the livers were harvested immediately, snap frozen in liquid nitrogen, and stored at -70°C until processed. In addition, blood was collected from the inferior vena cava, centrifuged for 10 minutes at 3000 rpm, and the plasma was subsequently frozen in liquid nitrogen and stored at -70°C.

Plasma lipid levels

Total cholesterol was determined by an enzymatic colorimetric assay using a kit supplied by Wako Di-

agnostics and Chemicals USA (Richmond, VA, USA). Plasma HDL-cholesterol, LDL-cholesterol, and triglycerides were determined using kits from Sigma Chemical Company (St. Louis, MO, USA) [11]. The plasma lipid concentrations from these animals have been previously reported [10].

Hepatic cholesterol content

Total, free, and esterified cholesterol content were quantified in hepatic tissue by Wako C and CII cholesterol kits, as described earlier (Wako Diagnostics and Chemicals,) [12].

Determination of LDL-R protein

Hepatic LDL-R protein abundance was determined in the plasma membrane preparation by Western blot analysis using a mouse antiovine LDL-R antibody (Cortex Biochem, Inc., Davis, CA, USA) as described previously [13].

HMG-CoA reductase protein determination

HMG-CoA reductase protein abundance in the liver extract was quantified by Western blot analysis as described by Ness et al [14] using a polyclonal anti-HMG-CoA reductase antibody (generously provided by Professor G.C. Ness, Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, Florida).

Determination of cholesterol 7 α -hydroxylase protein

Cholesterol 7 α -hydroxylase protein abundance in the liver tissue preparation was determined by Western blot analysis using a rabbit antirat cholesterol 7 α -hydroxylase antibody as described in our earlier studies [15]. The antibody employed in this assay was generously provided by Professor John Y.L. Chiang (Northeastern Ohio University College of Medicine, Rootstown, OH, USA).

Determination of ACAT-2 protein

Hepatic ACAT-2 protein abundance was quantified by Western analysis using a polyclonal antibody against ACAT-2 as described in our recent study [11]. The antibody employed in these experiments was a generous gift from Professor Lawrence L. Rudel, Department of Biochemistry and Comparative Medicine, Wake Forest University, Winston-Salem, North Carolina.

Determination of SR-B1 protein

Hepatic tissue HDL receptor abundance was determined in the liver tissue protein preparation by Western blot using a polyclonal HDL receptor antibody (Novus Biological, Inc., Littleton, CO, USA) and a horseradish peroxidase-linked antirabbit immunoglobulin G (IgG) (Amersham Life Science, Piscataway, NJ, USA) as described in our earlier study [16].

Table 1. Primers and probes used for the real time RT-PCR

Genes		DNA sequence	GeneBank Accession No.
HMG-CoA Reductase	F ^a	5'-CATGCTGCCAACATCGTCA-3'	X55286
	R ^b	5'-CCCACATTCTGTGCTGCATC-3'	
	P ^c	5'-6-FAM-TGCCATCTACATTGCATGTGCCCA-3'	
Cholesterol 7 α Hydroxylase	F ^a	5'-ACCTGCCGGTACTAGACAGCA-3'	NM.012942
	R ^b	5'-TGCGGATATTCAAGGATGCA-3'	
	P ^c	5'-6-FAM-CATCAAGGAGGCTCTGAGGCTTTCCA-3'	
LDL-R	F ^a	5'-CAACGGTGGCTGCCAGTAC-3'	NM.175762
	R ^b	5'-GAACTTGGGTGAGTGGGCAC-3'	
	P ^c	5'-6-FAM-TGTGCCTGCCTGCCCTCAGAT-3'	
SR-B1	F ^a	5'-AGGGTGTTTGAAGGCATCC-3'	NM.031541
	R ^b	5'-GACCCGTTGGCAAACAAAGT-3'	
	P ^c	5'-6-FAM-ACTTATCGCTTCACGGCCCCCG-3'	
ACAT2	F ^a	5'-CTATACTGCCAGGAGTGGTACGC-3'	AB075946
	R ^b	5'-AGCTCCCAAAATGTGCGCT-3'	
	P ^c	5'-6-FAM-CGGCGACACTGTCCCTTGCCC-3'	
18s rRNA	F ^a	5'-ACATCCAAGGAAGGCAGCAG-3'	X01117
	R ^b	5'-TCGTCACTACCTCCCCGG-3'	
	P ^c	5'-VIC-CGCGCAAATATCCCACTCCCGA-3'	

^a, Forward primer; ^b, reverse primer; ^c, probe.

Preparation of liver microsomes and activity assay for ACAT and HMG-CoA reductase

Frozen liver (400 mg) was homogenized in ice-cold buffer A containing 50 mmol/L Tris/HCl, pH 7.4, 250 mmol/L sucrose, 1 mmol/L EDTA, and protease inhibitors [1 mmol phenylmethylsulfonyl fluoride (PMSF), 10 μ g/mL aprotinin, 5 μ g/mL leupeptin, and 3 μ g/mL pepstatin]. Microsomes were then isolated by differential centrifugation, resuspended in buffer A, divided into several aliquots and stored at -70°C until used. Microsomal protein concentration was measured using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). Hepatic microsomal ACAT activity was quantified in a manner which was identical to that described in our earlier studies [11]. Hepatic microsomal HMG-CoA reductase abundance and activity were determined as described previously [17].

RNA preparation and real-time RT-PCR

Total RNA was prepared from frozen liver using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's procedures. RNA concentration was determined by spectrophotometry. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed with ABI 7700 real time PCR thermocycler using a kit from Applied Biosystems (Foster City, CA, USA). One tenth μ g of RNA was converted to cDNA by reverse transcription in 5 μ L of 5.5 mmol/L MgCl_2 , 0.5 mmol/L of each dNTPs, 2.5 μ mol/L of random hexamer, 0.4 U of RNase inhibitor, and 1.25 U of reverse transcriptase. The reaction mixture was incubated for reverse transcription for 10 minutes at room temperature

and subjected to one cycle consisting of 10 minutes at 25°C , 30 minutes at 48°C , and 5 minutes at 95°C .

PCR was done using AmpliTaq DNA polymerase in 25 μ L containing 5.5 mmol/L of MgCl_2 , 0.2 mmol/L of each dATP, dCTP, and dGTP, 0.4 mmol/L of dUTP, 0.25 U of AmpliTaq DNA polymerase, 5 μ L of reverse transcription mixture, and 0.2 μ mol/L of 5'- and 3'-primers, and 0.1 μ mol/L of 6-FAM-conjugated probe specific for HMG-CoA reductase, cholesterol 7 α -hydroxylase, LDL receptor, SR-B1, and ACAT-2. VIC-conjugated probe and primers specific for 18s rRNA were used as internal control. The primer sequences are summarized in Table 1. PCR amplification was done for 45 cycles in two-step cycling of denaturation at 94°C for 15 seconds, and annealing/extension at 60°C for 1 minute. The amplification process was analyzed and visualized using the Applied Biosystems Sequence Detector software (Applied Biosystems). The cycle number in the logarithmic phase (Ct value) was used to assess relative mRNA abundance in hepatic tissue of the study animals. Mean normalized gene expression was calculated using 18s rRNA expression as a reference [18].

Statistical analysis

Data were analyzed using Student *t* test. Values are reported as mean \pm SE with 6 rats per group unless otherwise indicated. Differences were considered statistically significant at $P < 0.05$.

RESULTS

General data

Data are summarized in Table 2. Compared to the LFCC group, the HFS group exhibited a 3.5-fold

Table 2. Body weight, systolic blood pressure, plasma total cholesterol (TC), LDL-C, VLDL-C, triglyceride (TG), TC-to-HDL-C ratio, and hepatic tissue TC and esterified cholesterol in rats given high-fat, high refined sugar (HFS) or low-fat, complex carbohydrate (LFCC) diets for 20 months

	LFCC	HFS
Body weight g	260 ± 6	374 ± 9 ^a
Systolic blood pressure mm Hg	123 ± 4	147 ± 4 ^a
TC mmol/L	1.49 ± 0.10	5.56 ± 0.58 ^a
LDL-C mmol/L	0.89 ± 0.06	3.45 ± 0.40 ^a
VLDL-C mmol/L	0.37 ± 0.07	1.53 ± 0.23 ^a
TG mmol/L	0.39 ± 0.04	2.58 ± 0.31 ^a
TC/HDL-C ratio	4.15 ± 0.30	5.30 ± 0.42 ^c
Liver TC mg/g	21.63 ± 0.89	27.13 ± 1.35 ^b
Liver esterified cholesterol mg/g	18.1 ± 0.80	21.98 ± 1.45 ^b

Values are mean and SE, *N* = 6 to 8 per group.

^a*P* < 0.001; ^b*P* < 0.01; ^c*P* < 0.05.

elevation of serum total cholesterol and a 6.5-fold rise in serum triglyceride concentration. This was coupled with a nearly 4-fold increase in serum LDL-cholesterol and VLDL-cholesterol concentrations, and a 50% rise in LDL/HDL cholesterol ratio in the HFS compared with the LFCC group.

Free and esterified cholesterol concentrations in the liver tissue were modestly elevated, and serum-to-liver cholesterol concentration ratio was 3.5-fold higher in the HFS animals compared to those in the LFCC group. Likewise, body weight was higher in the HFS than in the LFCC group.

HMG-CoA reductase and cholesterol 7 α -hydroxylase

Data are illustrated in Figures 1 and 2. HMG-CoA reductase mRNA abundance in the liver tissue of the HFS group was significantly higher than that in the LFCC group (*P* < 0.05). However, HMG-CoA reductase protein abundance and enzymatic activity were unchanged in the HFS group. Despite severe hypercholesterolemia, hepatic tissue cholesterol 7 α -hydroxylase mRNA and protein abundance were significantly lower in the HFS group than in the LFCC group (*P* < 0.01). Consequently, the HMG-CoA reductase-to-cholesterol 7 α -hydroxylase ratio, which reflects the balance between cholesterol synthetic and cholesterol catabolic capacities, was markedly increased in the HFS group.

LDL-R and SR-B1

Data are shown in Figures 3 and 4. Hepatic LDL-R protein abundance in the HFS group was significantly reduced compared to that in the LFCC group (*P* < 0.001). Although the mean value for hepatic LDL-R mRNA abundance in the HFS group was lower than that in the LFCC group, the difference did not reach statistical significance. In contrast to the LDL-R, hepatic SR-B1 protein abundance was significantly increased (*P* < 0.005), and SR-B1 mRNA abundance was insignificantly increased in the HFS group.

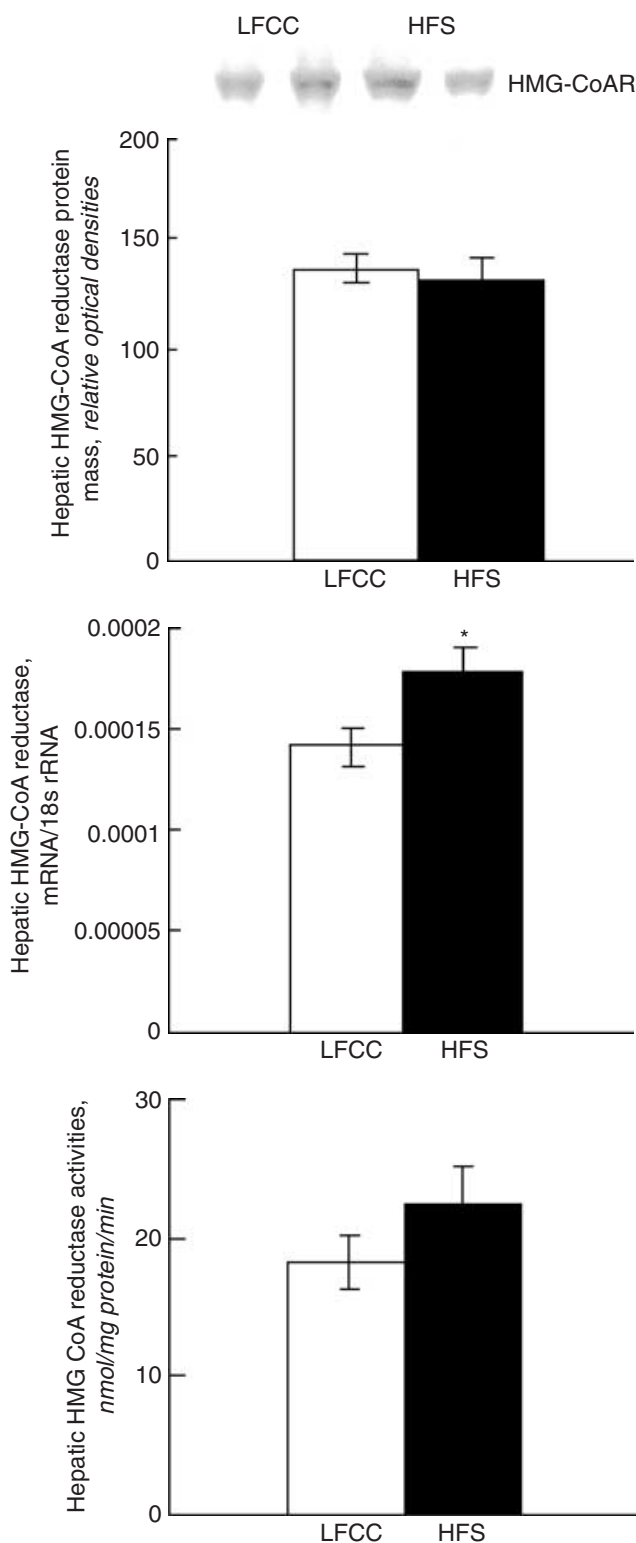


Fig. 1. Representative Western blot and group data depicting HMG-CoA reductase protein abundance, mRNA abundance, and enzymatic activities in liver of rats fed the low-fat, complex carbohydrate (LFCC) or high-fat, sucrose (HFS) diets. *N* = 6 per group. **P* < 0.05.

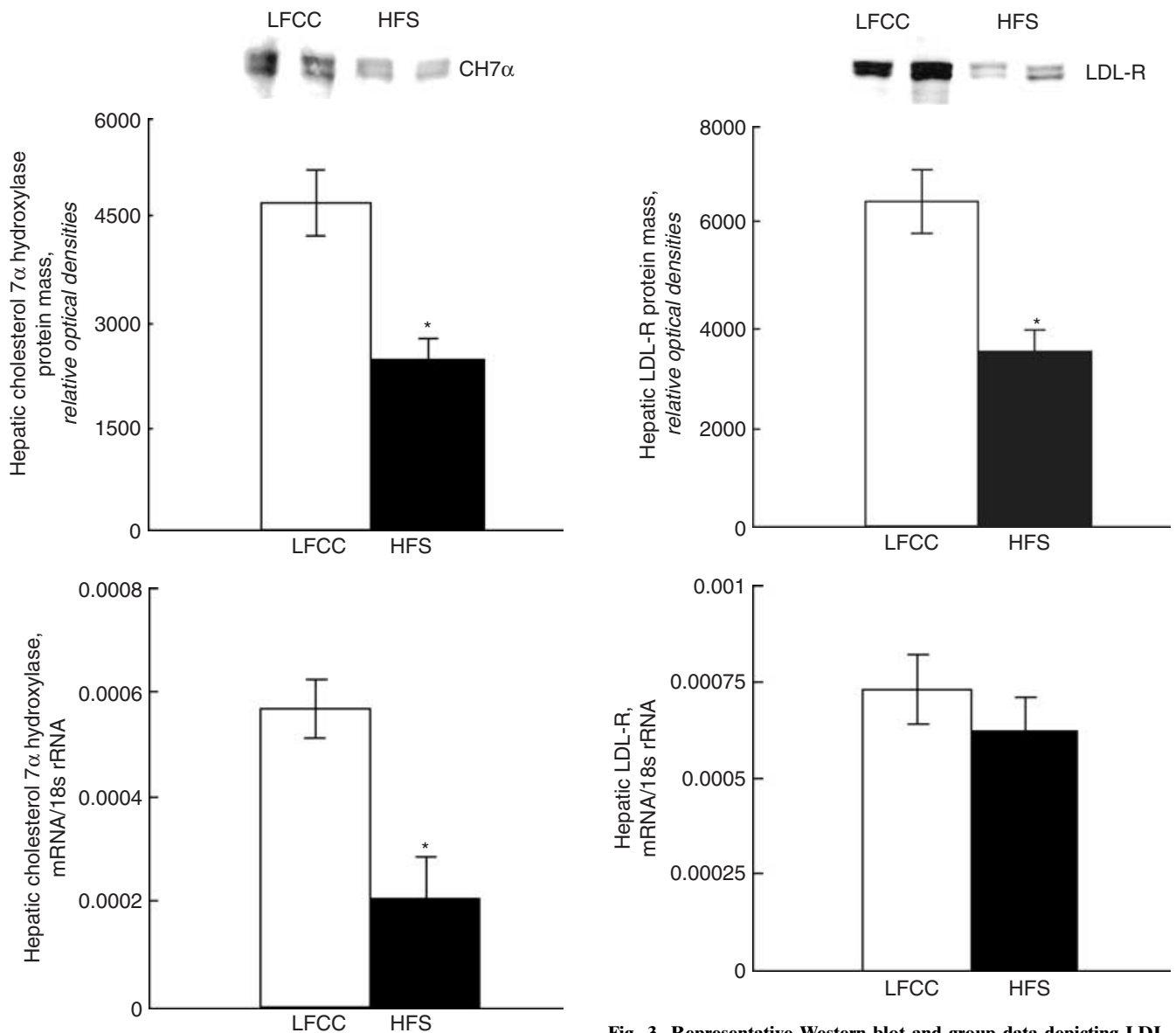


Fig. 2. Representative Western blot and group data depicting cholesterol 7 α -hydroxylase protein and mRNA abundance in liver of rats fed the low-fat, complex carbohydrate (LFCC) or high-fat, sucrose (HFS) diets. *N* = 6 per group. **P* < 0.01.

Fig. 3. Representative Western blot and group data depicting LDL-receptor (LDL-R) protein and mRNA abundance in liver of rats fed the low-fat, complex carbohydrate (LFCC) or high-fat, sucrose (HFS). *N* = 6 per group. **P* < 0.01.

ACAT-2

Data are depicted in Figure 5. Hepatic ACAT-2 protein abundance and ACAT activity were significantly elevated (*P* < 0.01), while ACAT-2 mRNA abundance was insignificantly increased in the HFS group compared to the LFCC group.

DISCUSSION

Hyperlipidemia is a major feature of the metabolic syndrome, and one of the cardinal risk factors for coronary artery disease. We have previously shown marked

hyperlipidemia, dysregulation of lipoprotein lipase and VLDL receptor deficiency in this model of diet-induced syndrome X [9]. The latter abnormalities could, in part, account for the associated hypertriglyceridemia and impaired clearance of VLDL and chylomicrons in diet-induced syndrome X. The present study was designed to investigate the effect of diet-induced syndrome X on protein expression of the key enzymes and receptors involved in hepatic cholesterol metabolism.

HMG-CoA reductase is an endoplasmic reticulum-bound and peroxisomal enzyme that is the rate-limiting step in cholesterol biosynthesis. While this enzyme is expressed in all tissues, it is more abundantly expressed

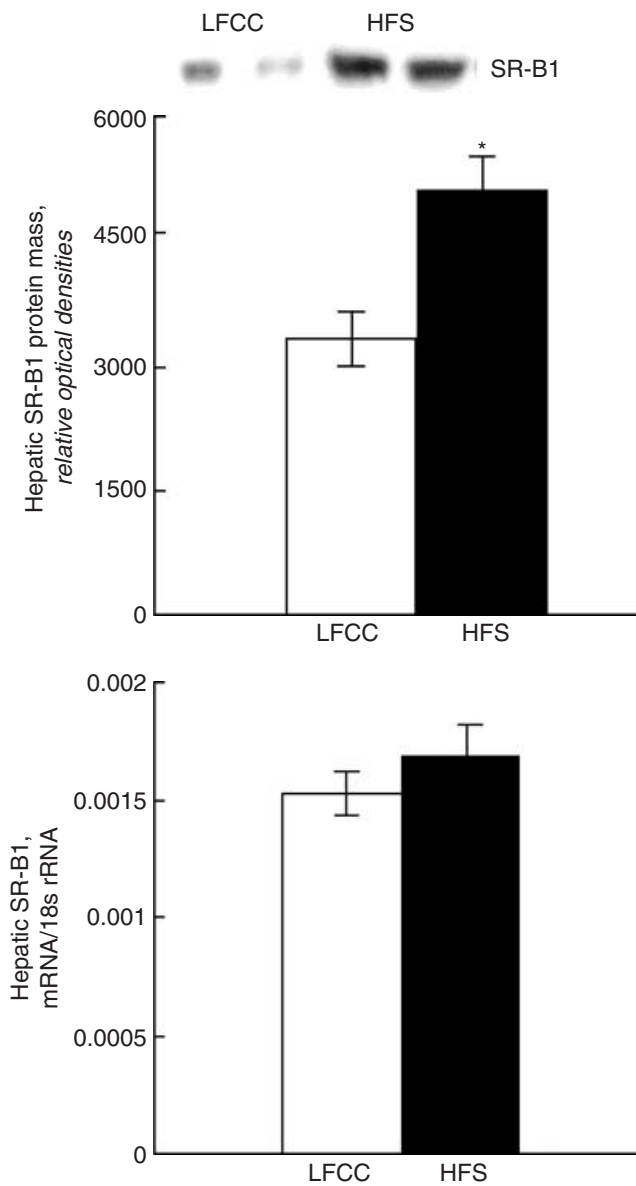


Fig. 4. Representative Western blot and group data depicting SR-B1 protein and mRNA abundance in liver of rats fed the low-fat, complex carbohydrate (LFCC) or high-fat, sucrose (HFS). $N = 6$ per group. $*P < 0.01$.

in the liver, which plays a central role in regulation of cholesterol metabolism and plasma cholesterol concentration [19]. In addition to its critical role in production, packaging, and secretion of cholesterol in the apoprotein B-containing lipoproteins, the liver is responsible for cholesterol catabolism to bile acids for disposal in the gut. The rate-limiting enzyme in cholesterol catabolism to bile acids is the unique cytochrome P450 enzyme, cholesterol 7 α -hydroxylase [20]. Thus, the balance between cholesterol biosynthesis and cholesterol catabolism by the liver is a critical determinant of serum cholesterol concentration. Despite severe hypercholesterolemia, hepatic

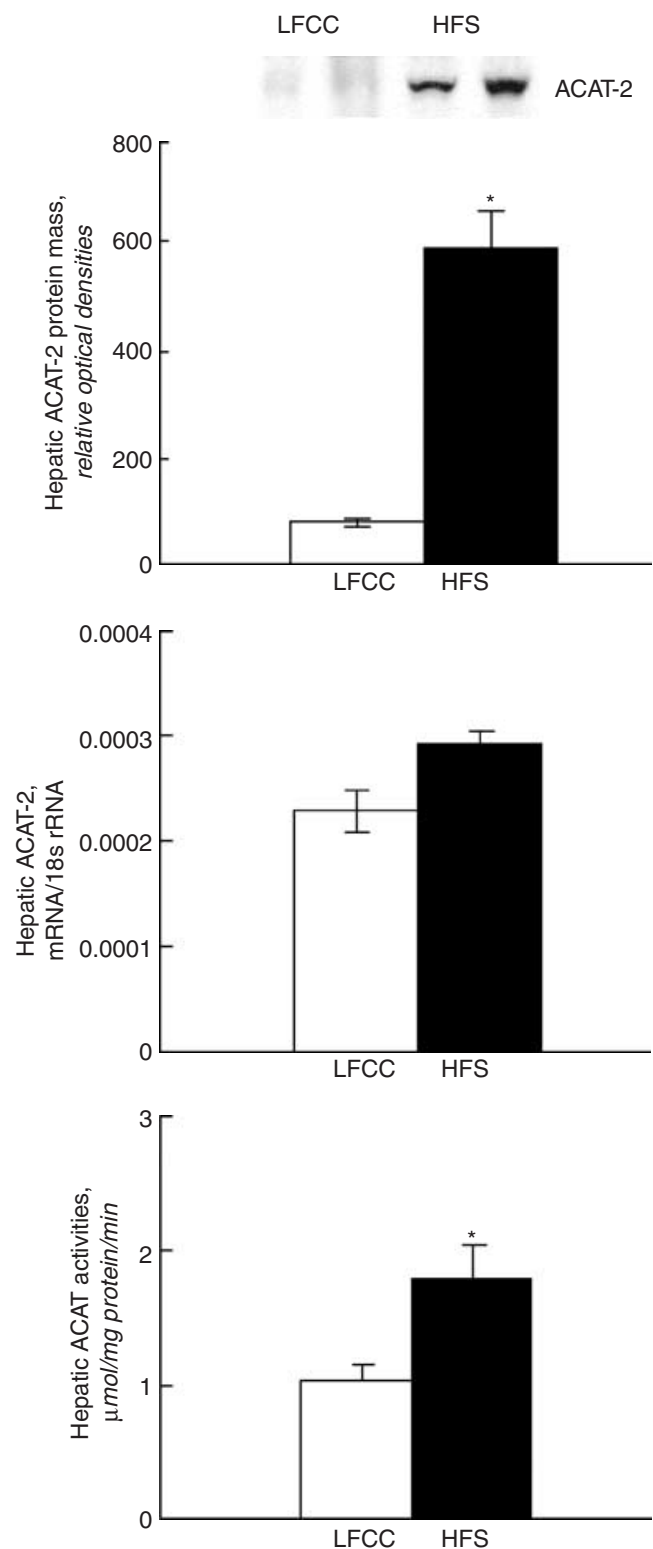


Fig. 5. Representative Western blot and group data depicting ACAT-2 protein and mRNA abundance, and ACAT activity in liver of rats fed the low-fat, complex carbohydrate (LFCC) or high-fat, sucrose (HFS). $N = 6$ per group. $*P < 0.01$.

HMG-CoA reductase protein abundance and activity were unchanged, and hepatic cholesterol 7 α -hydroxylase abundance was markedly reduced in HFS-fed animals. Thus, severe hypercholesterolemia in diet-induced syndrome X was associated with a more than two-fold rise in relative ratio of HMG-CoA reductase-to-cholesterol 7 α -hydroxylase. This observation points to relative reduction of cholesterol catabolism as a culprit in the pathogenesis of hypercholesterolemia in chronic, diet-induced syndrome X. It should be noted that in contrast to the high-fat sugar diet employed here, consumption of high-fat, high-cholesterol diets has been shown to down-regulate hepatic HMG-CoA reductase [21, 22]. These observations point to the role of excess exogenous cholesterol as a cause of down-regulation of hepatic HMG-CoA reductase in the latter studies. As with the present study, Vergara-Jimenez et al [23] reported no change in HMG-CoA reductase in male guinea pigs fed a high-fat, sucrose diet for 4 weeks. However, they found increased cholesterol 7 α -hydroxylase expression with short-term consumption of HFS diet in guinea pigs. The difference in cholesterol 7 α -hydroxylase expression with HFS diet between the present study and that found in the latter study may be due to differences in duration of intervention (4 weeks vs. 20 months), gender (males vs. females), and species used (guinea pig vs. rat). It is of note that diets high in either saturated or monounsaturated fat have been shown to mitigate up-regulation of cholesterol 7 α -hydroxylase mRNA abundance and activity in response to a high-cholesterol diet [24].

Plasma LDL concentration is a function of the rates of LDL synthesis and catabolism. HFS-fed animals exhibited a marked elevation of plasma LDL-cholesterol concentration. The primary pathway of cholesterol clearance by the liver is LDL-receptor-mediated uptake of cholesterol-rich LDL particles [25–27]. Elevation of serum total cholesterol and LDL-cholesterol in our HFS-fed group was associated with marked down-regulation of LDL-R protein expression. Liu et al [28] showed that consumption of a high-fat, high-cholesterol diet decreases hepatic LDL-receptor mRNA in mice. The reduction in LDL-receptor noted here is not due to increased cholesterol intake because the dietary cholesterol was normal in our HFS group [7]. However, the shared characteristic between the 2 diets was high-fat content, which must be, in part, responsible for the LDL-receptor deficiency found with both diets. In fact, studies by Srivastava et al [29] support this contention, as diets high in fat or fat and cholesterol depressed hepatic LDL-receptor mRNA in mice. Additionally, Hara et al [30], utilizing ¹²⁵I-labeled LDL, demonstrated that substituting saturated fat with complex carbohydrate increased the LDL binding to LDL-receptor and lowered plasma LDL-cholesterol. The biological significance of the LDL-receptor in cholesterol metabolism is illustrated by hereditary LDL-receptor

deficiency, which leads to severe hypercholesterolemia and premature atherosclerotic cardiovascular disease [31, 32]. Thus, because LDL-receptor protein content correlates well with LDL-receptor activity [33], acquired LDL-receptor deficiency in rats employed in the present study can contribute to elevations of serum total cholesterol and LDL-cholesterol concentrations in this model.

We have recently found significant down-regulation of VLDL-receptor in both skeletal muscle and adipose tissue, and marked reductions of lipoprotein lipase in the skeletal muscle of rats with diet-induced syndrome X [7]. Accordingly, the observed down-regulation of the VLDL-receptor and lipoprotein lipase is expected to contribute to the elevation of plasma VLDL in this model. We have further shown that chronic HFS feeding induces marked insulin resistance [7]. Insulin normally suppresses VLDL secretion [34], and insulin resistance is associated with the inability of insulin to suppress hepatic VLDL secretion [35, 36]. These events work in concert to increase the available pool of VLDL for eventual conversion to LDL.

In contrast to LDL, in which the receptor-mediated metabolism is well characterized, the precise regulation of HDL metabolism is not entirely understood. HDL mediates the removal of surplus cholesterol from extrahepatic tissues and its disposal in the liver, commonly referred to as “reverse cholesterol transport.” Acton et al [37, 38] isolated and cloned a cell membrane-associated protein that exhibited high-binding affinity for HDL. The authors identified this protein as a scavenger receptor class B, type 1 (SR-B1) molecule. Based on its high-binding affinity for HDL, SR-B1 was identified as an HDL receptor [39]. Transient HDL binding to its hepatic receptors results in removal and internalization of cholesterol esters from HDL-2 particles without degradation or uptake of the HDL apoprotein constituents [37, 38]. Hepatic SR-B1 abundance was increased in our rats with diet-induced syndrome X. The elevation of SR-B1 protein abundance in HFS-fed animals indicates that HDL-mediated hepatic cholesterol uptake may be relatively intact, contrasting severe reduction of LDL-receptor in this model. It would appear that an increase in SR-B1 activity would be desirable, since this may enhance the delivery of HDL cholesterol esters to the liver. The available data on the regulation of SR-B1 expression are limited. Spady et al [40] demonstrated that polyunsaturated fat increases SR-B1 expression, which may account for the HDL-reducing property of polyunsaturated fat diets. Hepatic over-expression of SR-B1 dramatically lowers plasma HDL and suppresses the development of atherosclerotic lesions in mice [41]. Interestingly, SR-B1 can avidly bind oxidized lipids [42], which are elevated in this model [8]. Consequently, the up-regulation of this scavenger receptor may be a compensatory response for the increase in oxidized lipids.

Liver ACAT-2 protein abundance and ACAT enzymatic activity were markedly elevated in rats fed the HFS diet. ACAT is an endoplasmic reticulum-bound enzyme that catalyzes intracellular esterification of cholesterol and formation of cholesterol ester in nearly all mammalian cells. Two isoforms of ACAT have been identified: ACAT-1, which is expressed in most tissues [43], and ACAT-2, which is primarily expressed in the liver and intestine [44–46]. Esterification of cholesterol by ACAT limits its solubility in the cell membrane lipids and promotes accumulation of cholesterol ester in the fat droplets within the cytoplasm. Thus, normal ACAT activity prevents potentially toxic accumulation of free cholesterol in various cell membranes. In addition, by modulating intracellular free cholesterol concentration, ACAT plays an important role in regulation of the cellular cholesterol signaling pathways [47]. Moreover, ACAT-mediated esterification of cholesterol plays an important role in packaging, production, and secretion of VLDL by the liver [48–50]. Similarly, by lowering the free cholesterol that regulates cholesterol 7 α -hydroxylase, ACAT modulates hepatic cholesterol catabolism [51], and thus, plasma cholesterol concentration. Thus, up-regulation of hepatic ACAT-2 abundance in the HFS-fed group can contribute to the observed elevation of serum VLDL concentration in this model. Hepatic esterified cholesterol content was increased in our HFS-fed animals, thus providing functional evidence for the observed increase in ACAT activity. Moreover, inhibition of ACAT has been shown to increase bile acid synthesis and cholesterol 7 α -hydroxylase expression [51]. Thus, up-regulation of hepatic ACAT may have contributed to the reduction of cholesterol 7 α -hydroxylase, and hence, hypercholesterolemia in this model.

It is of note that the magnitude of the changes in protein abundance or activities of some of the measured molecules did not match the changes seen in the corresponding mRNA abundance. This phenomenon illustrates the fact that the given proteins are separately regulated at multiple levels and are subject to transcriptional, translational, and post-translational regulation.

CONCLUSION

Severe dyslipidemia in rats with chronic, diet-induced syndrome X is associated with marked down-regulations of hepatic cholesterol 7 α -hydroxylase and LDL-receptor, coupled with marked up-regulation of hepatic ACAT and elevated HMG-CoA reductase-to-cholesterol 7 α -hydroxylase ratio. Dysregulation of these important lipid regulatory proteins, together with the previously demonstrated down-regulations of lipoprotein lipase and VLDL receptor, contributes to altered lipid metabolism in diet-induced syndrome X.

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